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A DEMONSTRATION OF PHOTOSYNTHESIS

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The difficulties in the way of a satisfactory demonstration of photosynthesis in land plants are too well known to require comment. They are greatest when quantitative results are desired. It is, of course, precisely these which are most important.

It is taken for granted that the reader is familiar with methods now in use.[1] The chief requisites appear to be (1) a method of removing at intervals satisfactory samples of the gases by which the leaf is surrounded (to accomplish this it is necessary to stir and mix the gases before taking the sample). (2) A method of gas analysis, simple and sufficiently accurate. (3) It is desirable to avoid the use of mercury, since the leaf is easily injured by mercury vapor.

The simple apparatus here described seems to meet these requirements and is easily constructed and kept in order. Its special advantage is that it permits the mixing of gases and the withdrawal of samples at will. In this way the progress of photosynthesis can be followed and the dynamics of the reaction investigated. It is also possible, when studying photosynthesis, to determine respiration without removing the leaf from the apparatus or changing the gases which surround it.

The apparatus consists of a wide-mouthed bottle (figure 1) or jar (the larger the better, up to a capacity of one gallon) with a stopper perforated by three or more short glass tubes (*R*). Each of these is connected (by rubber tubing) to a short glass tube (*S*) directly above it. This is in turn connected (by rubber tubing) to a tube about two feet long, (*A*), which is held in a vertical position by an arm (*T*) of a ring-stand. This tube should be of at least 9 mm.

[1] Cf. Ganong, W. F. Plant Physiology, 107 ff. 1908.

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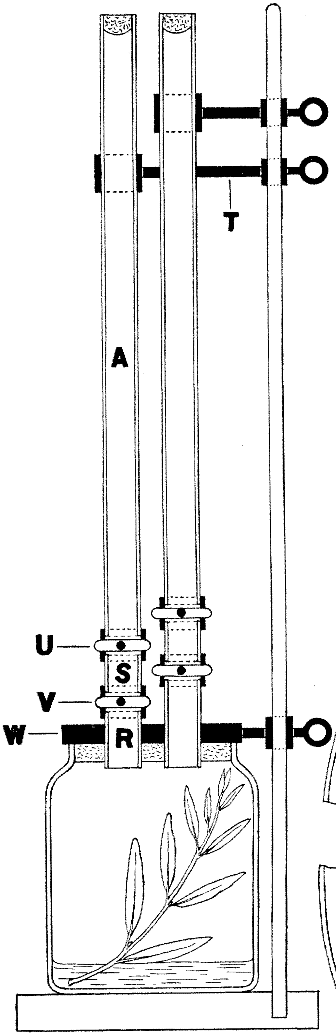

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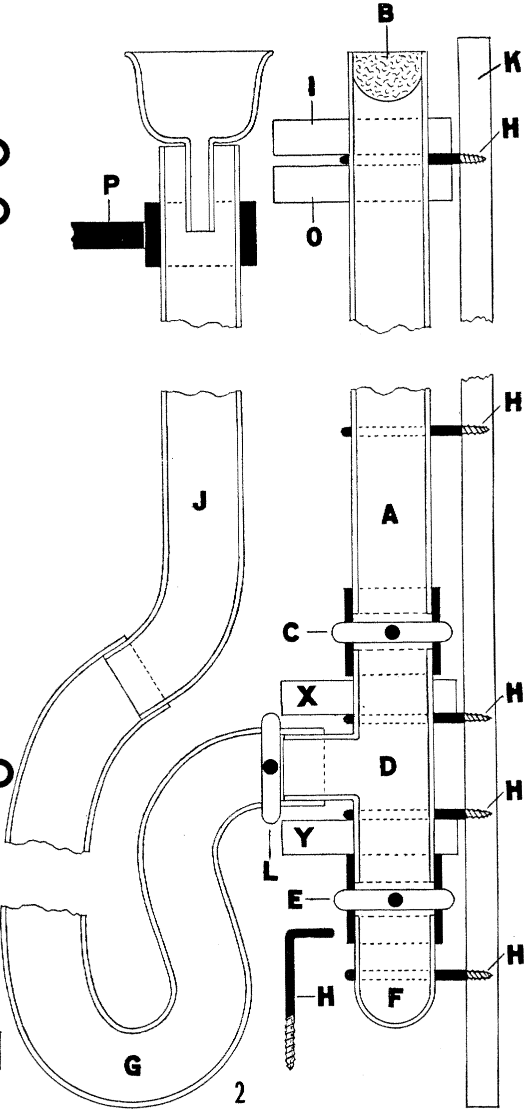

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FIG. 1. Apparatus for the demonstration of photosynthesis. Samples of the gas (after thorough mixing by means of the water in the bottle) are removed in the analysis tubes (*A*).

FIG. 2. Apparatus for gas analysis. The gas is contained in *A*; *D* is filled with potassium hydrate and *F* with pyrogallol.

internal diameter. A ring (*W*) is fastened firmly against the stopper so that the stand may be inverted without displacing any part of the apparatus.

The procedure is as follows: We place vigorous plants[1] (covered with young leaves capable of active photosynthesis) in the bottle and fill it with water to about one tenth of its capacity. The stopper is then inserted. If provision has been made for three long tubes only one of them is now attached. One of the short tubes which perforate the stopper is connected to a CO_2-generator[2] (this tube is first clamped off). The other short tube is connected to a piece of glass tubing of small diameter.

The apparatus is now inverted, allowing the long tube to fill with water. Sufficient CO_2 is now admitted to drive out most of the water from the bottle through the narrow tube.[3] The apparatus is now returned to the upright position and the two remaining long tubes are connected to the bottle.

The apparatus is now inverted, allowing each of the long tubes to become partially filled with water. On placing in an upright position the water runs back into the bottle. This is repeated until the gases are well mixed and the water is nearly in equilibrium with them.

In case it is desirable to protect the leaf from wetting during the inversion of the apparatus the plant may be tied to a vertical support (passing downward through the center of the stopper) in such fashion that it does not touch the sides of the bottle.

By inverting the apparatus just enough water is allowed to run into one of the tubes, *A*, so that when in the upright position the water level can be seen above *U*. The tube *A* is then closed by two clamps, *U* and *V*. The apparatus is placed upright and the tube *A* is removed by cutting the rubber tubing below *U* or by slipping it off from *S*. This allows the removal of *A* without admitting air to *A* or to the bottle (the tube *A* being closed by the clamp *U* and the tube *R* by the clamp *V*).

[1] *Tradescantia* may be recommended for this purpose, especially kinds with non-striped leaves.

[2] The CO_2 must be well washed on its way to the bottle.

[3] Another procedure is to put into the bottle only enough water to fill two long tubes. Keeping the apparatus upright, run in CO_2 allowing it to displace air which issues through another short tube and is collected in an inverted graduate filled with water and dipping into water. When air representing about one tenth of the capacity of the bottle has been displaced by CO_2 the long tubes are put into place.

Care should be taken not to grasp the tube *A* directly, since this may warm the gas within it. It may be convenient to attach two spring clothes pins (one at either end) by which it may be handled.

The tube *A* is shown on a larger scale in figure 2: it is closed by a rubber stopper *B* (which may be shaped like a meniscus, as shown in the figure, though this is by no means necessary).

The tube is placed on the meter stick *K*, where it is held in place by "sash curtain hooks" *H*. These are pieces of metal bent at right angles, with a screw thread at one end. They are turned outward before the tube is in place. Then they are turned inward to hold it. The tube can be held in any desired position by attaching to it spring clothes pins (*I*, *O*) on each side of the sash curtain hooks. In consequence the tube is held firmly in place.

We now attach the rest of the apparatus. This consists of a T-tube *D*, a tube *F* (which is closed at one end), and a longer tube, *J*. All of these have a diameter as large as that of *A* (or larger). They are connected by two short pieces of rubber tubing furnished with clamps (at *C* and *E*) and a longer piece, *G* (with clamp at *L*). The tube *F* (which should be of the same diameter as *A* and at least 1 inch long) is filled with 20 percent aqueous pyrogallol[5] (taking care to exclude air), after which it is clamped off at *E* and the rubber connection *thoroughly* rinsed before being attached to *D* so that no pyrogallol can enter *D*.

The apparatus is placed on the meter stick and fastened by the hooks before being attached to the tube *A* (by means of the rubber tubing at *C*). It is very important that the tubes *A* and *D* be firmly attached to the meter stick so that they can not be pulled apart, allowing the reagents to escape.

A thistle tube is now placed in *J*, which is firmly fastened upon the retort stand by the clamp, *P*, so as to hang vertically. The meter stick with attachments is now inverted several times to make sure that the tubing is firmly attached to the meter stick and that all joints are secure. It is then hung on the retort stand in such a manner as to be easily detached. After opening the clamp at *L* we pour into the thistle tube 20 percent KOH until *D* is filled and the level appears

[5] This strength of pyrogallol will quickly (if above 15° C.) absorb at least 15 times its volume of oxygen. Hence if *F* is an inch long and filled with pyrogallol it can easily absorb at least 15 inches of oxygen in *A*. Ordinarily there are less than 8 inches of oxygen in *A*.

in *J*. The rubber tubing above *E* is squeezed to remove air. The meter stick is then inverted (without disturbing the tube *J*) and the rubber tubing at *C* is squeezed to remove air. When the air is all out of *D* the clamp at *L* is closed. The meter stick is returned to its upright position (as shown in the figure). The clamp *L* is then opened slightly so that when the rubber tubing above *E* is squeezed a movement of the liquid is seen in *J*. The clamp *L*, in this condition, permits the free movement of water while affording an effective barrier against the passage of a large gas-bubble.

The tubes are now adjusted so that the surface of the liquid is at the same level in *A* as in *J*. The position of the bottom of the stopper *B* is read off on the meter stick (the spring clothes pins, *O*, *I*, will hold it firmly in this position if properly adjusted). The clamp *C* is now opened somewhat, to secure atmospheric pressure in *A*, and the position of the meniscus at once read off on the meter stick (before lye has had time to diffuse into the tube above *C*). This gives the length of the gas column.

The clamp *C* is now fully opened and moved out of the way and the meter stick inverted (without disturbing the tube *J*). In doing this the operator should grasp the meter stick at the ends and avoid touching the glass tubing. If the glass tubing is handled the gas may be warmed by the contact and the tubes may be pulled apart at the joints (since the rubber tubing becomes slippery from the lye). There is some strain on the joints at *L* and at the end of *J*; it is very important that they be firmly secured by winding with wire or string (or they may be firmly held by means of spring clothes pins). As these are permanent connections it may be advisable to heat the glass before slipping the rubber tubing over it. On cooling the rubber adheres firmly to the glass. There is little or no strain on the other joints, but they should be secured as a matter of precaution.

When the meter stick is inverted the side neck of the T-tube should always point downward so as to prevent gas from entering it.

The rubber tube *G* should be kept in such a position that it never kinks in such a manner as to prevent free passage of liquid.

After inverting the meter stick several times in succession we restore it to the usual position and raise or lower the tube *A* until the liquid stands at the same level in *A* and in *J* (it may be necessary to pour more water into *J*). The length of the gas column in *A* is then read on the meter stick. The shrinkage, divided by the original length and multiplied by 100, gives the percent of CO_2.

After the reading is taken we invert the meter stick several times and take another reading. This should be continued until two successive readings agree.

We now open the clamp at *E*, allowing the pyrogallol to enter and absorb the oxygen. The procedure is the same as for CO_2. The shrinkage in the length of the column, due to absorption by pyrogallol, divided by the original length of the column and multiplied by 100 gives the percent of O_2.

If the apparatus were closed during the absorption by KOH and by pyrogallol there would be a tendency to suck in air at the joints. This is prevented by maintaining a passage for water through the clamp *L*, which is kept slightly open for this purpose.

It may be added that all the tubes (whether glass or rubber) must be large enough to permit gas to pass freely into them and displace water, and that *J* must be large enough to be easily filled with liquid through the thistle tube. An internal diameter of 10 mm. will be found sufficient.

In order to clean the apparatus it should be placed in running water under the tap and disconnected at *C* and *E*.

A slender tube of metal or glass should be connected to the tap and inserted to the very bottom of *A*, so as to rinse it thoroughly.

Students should practice analyzing the laboratory air for oxygen until the results are correct to within at least 2 percent. If the gas column is 600 mm. long 2 percent is 12 mm. on the tube. It is easy to read to .1 or 2 mm. (parallax must be avoided).

Since all the work is done at laboratory temperature (the reagents being at the same temperature) no correction is needed.

As we now know the composition of the gas at the start, we may expose the plant to sunshine (for several days in succession if desired) and again analyze the gas by removing another of the long tubes. If photosynthesis has not progressed satisfactorily another exposure may be made and the gas subsequently analyzed by removing another tube. After all the tubes are removed new tubes (filled with air or with water) may be attached, a correction being made for the air or water thus added to the system.

When the plant is exposed to sunshine the gas is heated and tends to escape at the joints: on cooling air may be sucked in. To prevent this (and to prevent diffusion of CO_2 through the rubber) all the rubber tubing should be coated with paraffin.

In addition a water seal may be provided by tying a piece of oilcloth around the neck of the bottle so as to project in the form of a tube above it. This tube should be filled with water until all the joints are submerged. Or the whole apparatus may be placed in a large jar filled with water. These precautions will not be necessary if the joints are made secure by heating the glass before the rubber is slipped on, so as to make a permanent union on cooling.

It is important to have a control (containing no plants) which is kept beside the apparatus containing the plant. It is also desirable to have a control containing plants which is kept in the dark.

Respiration may be studied in precisely the same way as photosynthesis (it is not necessary in this case to add CO_2 at the start).

The method here outlined permits us to follow the progress of photosynthesis by making analyses at frequent intervals. In this way a time curve of photosynthesis may be plotted in order to study the dynamics of the process.

The influence of reagents (anesthetics, etc.) on respiration and photosynthesis may be studied advantageously by this method. It may be added that it has likewise been useful in studying the respiration of animals.

Summary

An apparatus for the demonstration of photosynthesis is described which permits:

1. Removal at intervals of satisfactory samples of the gases by which the leaf is surrounded.

2. Stirring and mixing of the gases when necessary.

3. Analysis of the gases by a simple method which is sufficiently accurate for ordinary purposes.

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